FORM PTO-1390 (Modified) (REV 1-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **101195-44**

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): **09/786725**

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/DE99/02816	**3 September 1999 (03.09.99)**	**8 September 1998 (09.09.98)**

TITLE OF INVENTION

Diagnostic Method for Detecting Disturbances of the Pancreas

APPLICANT(S) FOR DO/EO/US

Hans-Werner Heinrich; Rainer Kleinert; Udo Meyer; and Heinz-Jurgen Wagner

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ A copy of the International Search Report (PCT/ISA/210).
8. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☐ Other items or information:

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/786725	PCT/DE99/02816	101195-44

21. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO	**$1,000.00**
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO	**$860.00**
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$710.00**
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$690.00**
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)	**$100.00**
	ENTER APPROPRIATE BASIC FEE AMOUNT =	**$860.00**
	Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$130.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	18 - 20 =	0	x $18.00	**$0.00**
Independent claims	7 - 3 =	4	x $80.00	**$320.00**
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**
	TOTAL OF ABOVE CALCULATIONS =			**$1,310.00**
	Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable)**. ☒			**$655.00**
	SUBTOTAL =			**$655.00**
	Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +			**$0.00**
	TOTAL NATIONAL FEE =			**$655.00**
	Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐			**$0.00**
	TOTAL FEES ENCLOSED =			**$655.00**
			Amount to be: refunded	$
			charged	$

☐ A check in the amount of to cover the above fees is enclosed.

☒ Please charge my Deposit Account No. **14-1263** in the amount of **$655.00** to cover the above fees.
A duplicate copy of this sheet is enclosed.

☐ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

the correspondence address associated with Customer Number 27387

27387
PATENT TRADEMARK OFFICE

SIGNATURE

Bruce S. Londa
NAME

33-531
REGISTRATION NUMBER

March 8, 2001
DATE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
Atty's Docket No. 101195-44

APPLICANT : Hans-Werner Heinrich et al.

FILED : Concurrently Herewith

FOR : Diagnostic Procedure for Recognition of Functional Disorder of the Pancreas

PRELIMINARY AMENDMENT

Hon. Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Prior to examination, please amend the application as follows:

IN THE SPECIFICATION

Page 1, after line 2, please insert --Background of the Invention--;

Page 2, after line 23, please insert --Summary or the Invention--.

IN THE CLAIMS

Please amend the claims originally amended under Article 36 according to the attached marked-up pages. A clean copy of the

amended claims is also enclosed.

REMARKS

The above amendments were made to place the application into proper United States Patent Format.

Respectfully Submitted,

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Marked-up Amended Claims

Patent Claims (amended under PCT Chapter II)

1. Diagnostic procedure for identification of a disorder of the pancreas by determining the overall content of all known pancreatic elastases (iso-enzymes)in the serum, secretions or excretions of a patient.

2. (amended) Procedure according to claim 1, ~~characterised by~~ wherein the fact that identification is by means of immuno-chemical systems using monclonal or polyclonal antibodies that can singly and specifically or cross-reactively recognise all elastase iso-enzymes with the amino-acid sequence Thr-Met-Val-Ala-Gly-Gly-Asp-Ile-Arg.

3. (amended) Procedure according to claim 2, ~~characterised by~~ wherein the fact that the antibodies used are obtained by means of antigenes consisting of the complete elastases 1, 2 and 3 or of their sub-units. The amino-acid sequence Thr-Met-Val-Ala-Gly-Gly-Asp-Ile-Arg is not used as an elastase fragment or as an immunologically effective partial sequence thereof.

4. (amended) Procedure according to claim 3, ~~characterised by~~ wherein the fact that the following synthetic peptides are primarily used as antigenes which after the immunisation of animals induce antibodies which cross-reactively recognise several elastases but do not concern the amino-acid sequence Thr-Met-Val-Ala-Gly-Gly-Asp-Ile-Arg:

NH2-A-V-K-E-G-P-E-Q-V-I-P-I-N-COOH
NH2-Y-T-N-G-P-L-P-D-K-L-Q-Q-A-R-COOH
NH2-R-S-G-C-N-G-D-S-G-G-P-L-N-COOH
NH2-G-P-L-N-C-P-T-E-D-G-G-W-Q-COOH

NH2-G-T-E-A-G-R-N-S-W-P-S-Q-I-COOH
NH2-H-N-L-S-Q-N-D-G-T-E-Q-Y-V-COOH
NH2-W-G-K-T-K-T-N-G-Q-L-A-COOH
NH2-V-S-S-R-G-C-N-V-S-R-K-P-T-COOH
NH2-G-G-E-E-A-R-P-N-S-W-P-W-Q-COOH
NH2-S-S-S-R-T-Y-R-V-G-L-G-R-H-N-COOH
NH2-K-D-W-N-S-N-Q-I-S-K-G-N-D-COOH
NH2-G-P-L-N-C-Q-A-S-D-G-R-W-COOH
NH2-G-A-L-P-D-D-L-K-Q-G-R-L-COOH
NH2-S-L-Q-Y-E-K-S-G-S-F-Y-COOH
NH2-F-G-C-N-T-R-R-K-P-T-V-F-T-COOH

5. Procedure according to ~~claims 1 - 5, characterised by~~ claim 1, wherein the fact that antibodies are used singly or in a combination in an immuno-chemical identification system.

6. (amended) Immunological test kits for the diagnosis and progress check of diseases of the pancreas using stool or body fluids containing one or more of the antibodies used in ~~claims 2 - 5~~ claim 2.

7. Procedure for obtaining monoclonal and/or polyclonal antibodies that react specifically with human elastase 1 in stool or body fluids and are induced by the usual immunisation procedures, characterised by the fact that the peptides A-V-K-E-G-P-E-Q-V-I-P-I-N, Y-T-N-G-P-L-P-D-K-L-Q-Q-A-R, R-S-G-C-N-G-D-S-G-G-P-L-N and G-P-L-N-C-P-T-E-D-G-G-W-Q or immunogenic partial peptides thereof are used as antigenes for the immunisation of vertrebrates, especially of small mammals and birds.

8. (amended) Procedure according to claim 7, ~~characterised by~~ wherein the fact that before immunisation the free peptides are coupled with suitable carrier substances, primarily haemoczanine or albumin.

9. (amended) Procedure according to ~~claims 7 and 8, characterised by~~ claim 7, wherein the fact that polyclonal antibodies are produced using chickens as experimental animals.

10. (amended) Polyclonal antibodies, insofar as they were produced according to ~~claims 7 to 9~~ claim 7.

11. (amended) Monoclonal antibodies, insofar as they were produced according to ~~claims 7 and 8~~ claim 7.

12. Peptide A-V-K-E-G-P-E-Q-V-I-P-I-N

13. Peptide Y-T-N-G-P-L-P-D-K-L-Q-Q-A-R

14. Peptide R-S-G-C-N-G-D-S-G-G-P-L-N

15. Peptide G-P-L-N-C-P-T-E-D-G-G-W-Q

16. (amended) Purification and detection systems for human elastase 1, characterised by the fact that they contain at least one of the invention antibodies according to ~~claims 10 and 11~~ claim 10 or 11.

17. Immunological test kits for the diagnosis and progress check of diseases of the pancreas and of mucoviscidosis using stool or body fluids.

18. (amended) Immunological test kits according to claim 17, ~~characterised by~~ wherein the fact that 2 different antibodies are used (Sandwich-ELISA).

Clean Copy - Amended Claims

Patent Claims (amended under PCT Chapter II)

1. Diagnostic procedure for identification of a disorder of the pancreas by determining the overall content of all known pancreatic elastases (iso-enzymes)in the serum, secretions or excretions of a patient.

2. (amended) Procedure according to claim 1, wherein the fact that identification is by means of immuno-chemical systems using monclonal or polyclonal antibodies that can singly and specifically or cross-reactively recognise all elastase iso-enzymes with the amino-acid sequence Thr-Met-Val-Ala-Gly-Gly-Asp-Ile-Arg.

3. (amended) Procedure according to claim 2, wherein the fact that the antibodies used are obtained by means of antigenes consisting of the complete elastases 1, 2 and 3 or of their sub-units. The amino-acid sequence Thr-Met-Val-Ala-Gly-Gly-Asp-Ile-Arg is not used as an elastase fragment or as an immunologically effective partial sequence thereof.

4. (amended) Procedure according to claim 3, wherein the fact that the following synthetic peptides are primarily used as antigenes which after the immunisation of animals induce antibodies which cross-reactively recognise several elastases but do not concern the amino-acid sequence Thr-Met-Val-Ala-Gly-Gly-Asp-Ile-Arg:

NH2-A-V-K-E-G-P-E-Q-V-I-P-I-N-COOH
NH2-Y-T-N-G-P-L-P-D-K-L-Q-Q-A-R-COOH
NH2-R-S-G-C-N-G-D-S-G-G-P-L-N-COOH
NH2-G-P-L-N-C-P-T-E-D-G-G-W-Q-COOH

NH2-G-T-E-A-G-R-N-S-W-P-S-Q-I-COOH
NH2-H-N-L-S-Q-N-D-G-T-E-Q-Y-V-COOH
NH2-W-G-K-T-K-T-N-G-Q-L-A-COOH
NH2-V-S-S-R-G-C-N-V-S-R-K-P-T-COOH
NH2-G-G-E-E-A-R-P-N-S-W-P-W-Q-COOH
NH2-S-S-S-R-T-Y-R-V-G-L-G-R-H-N-COOH
NH2-K-D-W-N-S-N-Q-I-S-K-G-N-D-COOH
NH2-G-P-L-N-C-Q-A-S-D-G-R-W-COOH
NH2-G-A-L-P-D-D-L-K-Q-G-R-L-COOH
NH2-S-L-Q-Y-E-K-S-G-S-F-Y-COOH
NH2-F-G-C-N-T-R-R-K-P-T-V-F-T-COOH

5. Procedure according to claim 1, wherein the fact that antibodies are used singly or in a combination in an immunochemical identification system.

6. (amended) Immunological test kits for the diagnosis and progress check of diseases of the pancreas using stool or body fluids containing one or more of the antibodies used in claim 2.

7. Procedure for obtaining monoclonal and/or polyclonal antibodies that react specifically with human elastase 1 in stool or body fluids and are induced by the usual immunisation procedures, characterised by the fact that the peptides A-V-K-E-G-P-E-Q-V-I-P-I-N, Y-T-N-G-P-L-P-D-K-L-Q-Q-A-R, R-S-G-C-N-G-D-S-G-G-P-L-N and G-P-L-N-C-P-T-E-D-G-G-W-Q or immunogenic partial peptides thereof are used as antigenes for the immunisation of vertrebrates, especially of small mammals and birds.

8. (amended) Procedure according to claim 7, wherein the fact that before immunisation the free peptides are coupled with suitable carrier substances, primarily haemoczanine or albumin.

9. (amended) Procedure according to claim 7, wherein the fact that polyclonal antibodies are produced using chickens as experimental animals.

10. (amended) Polyclonal antibodies, insofar as they were produced according to claim 7.

11. (amended) Monoclonal antibodies, insofar as they were produced according to claim 7.

12. Peptide A-V-K-E-G-P-E-Q-V-I-P-I-N

13. Peptide Y-T-N-G-P-L-P-D-K-L-Q-Q-A-R

14. Peptide R-S-G-C-N-G-D-S-G-G-P-L-N

15. Peptide G-P-L-N-C-P-T-E-D-G-G-W-Q

16. (amended) Purification and detection systems for human elastase 1, characterised by the fact that they contain at least one of the invention antibodies according to claim 10 or 11.

17. Immunological test kits for the diagnosis and progress check

of diseases of the pancreas and of mucoviscidosis using stool or body fluids.

18. (amended) Immunological test kits according to claim 17, wherein the fact that 2 different antibodies are used (Sandwich-ELISA).

PCT

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Internationales Büro

INTERNATIONALE ANMELDUNG VERÖFFENTLICHT NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT)

(51) Internationale Patentklassifikation 7 :		(11) Internationale Veröffentlichungsnummer: **WO 00/14542**
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Veröffentlicht

Ohne internationalen Recherchenbericht und erneut zu veröffentlichen nach Erhalt des Berichts.

(54) **Title:** DIAGNOSTIC METHOD FOR DETECTING DISTURBANCES OF THE PANCREAS

(54) **Bezeichnung:** DIAGNOSTISCHES VERFAHREN ZUR ERKENNUNG EINER PANKREASFUNKTIONSSTÖRUNG

(57) **Abstract**

Disclosed is a method for detecting disturbances in the functioning of the pancreas, whereby parts of all elastase isoenzymes produced in the pancreas and synthetic amino acid sequences are used as antigens to obtain specific antibodies. The invention also relates to the use of said antibodies in immunochemical test methods.

(57) **Zusammenfassung**

Es wird ein Verfahren zur Erkennung einer Pankreasfunktionsstörung beschrieben. Das Ziel wird erfindungsgemäß durch die Verwendung von Teilen aller Isoenzyme der Pankreas–Elastase und synthetischer Aminosäuresequenzen als Antigene für die Gewinnung spezifischer Antikörper und deren Verwendung in immunchemischen Testverfahren erreicht.

Diagnostic Procedure for Recognition of Functional Disorder of the Pancreas.

The invention relates to a diagnostic procedure for the recognition of a functional disorder of the pancreas. The areas of application are medicine and the pharmaceutical industry.

Functional disorders of the pancreas may be the result of various illnesses whose diagnosis should be underpinned by the definition of functional criteria. The most frequent diseases of the pancreas are chronically recurrent and acute pancreatitis.

Chronic pancreatitis is an insidious progressive disease in which functioning pancrease tissue gradually degenerates in a scleroticising process. It is characterised by its clinical symptoms (abdominal complaints, steatorrhoea, weight loss), typical morphological changes of the gland (calcification, dilated and irregularly demarcated Ductus pancreaticus) and by a progressive exocrine and endocrine loss of function (indigestion, diabetes mellitus). The incidence of chronic pancreatitis is 6 or 8 new cases per 100 000 persons per annum in West Europe. The diagnosis of chronic pancreatitis is becoming increasingly frequent as a result of increasing alcohol consumption.

A number of functional criteria can be identified for the underpinning of clinical and morphological diagnoses. The most sensitive method of analysis is the Secretin-Caerulin or Secretin-Cholecystocinin test, which is, however, unpleasant for the patient and very time-consuming. Indirect test methods that are used include the Lundh-, NBT-PABA- and Pancreoauryl-tests. The identification of trypsin in serum or of chymotrypsin in stool are also of a certain practical significance. The disadvantage that is common to all these indirect test methods is

their lack of specificity.
Of all the indirect function tests, the identification of Elastase 1 is the test with the highest sensitivity and specificity (Löser,C., Therapie & Erfolg 1997; 1:411-413). It has become established in daily practice as the standard test for exocrine pancreas function diagnostics. The basis for this test consists of polyclonal antibodies against Elastase 1 (Elastase 1-RIA, Abbott) or mono- and/or polyclonalen anti-Elastase-1 antibodies, which are obtained by immunisation with an antigen containing the amino-acid sequence Thr-Met-Val-Ala-Gly-Gly Asp-Ile-Arg or immunologically effective partial peptides of this. (EP 0 547 059 B1).

Pancreas-Elastase is a proteolytic digestion enzyme. Compared with the common parameters of pancreas diagnostics (e.g. chymotrypsin activity in stool), quantitative Elastase identification has crucial advantages. The enzyme is formed exclusively in the pancreas and displays extraordinary stability during the passage through the intestines, i.e. the concentration of Elastase reflects the secretion performance of the pancreas.

Despite its superiority to other exocrine parameters, the traditional Elastase 1-ELISA methods fails in too many cases to identify pancreas elastase, even though it is present in substantial concentrations.

The object of the invention is therefore to develop a more sensitive procedure for the recognition of a functional disorder of the pancreas on the basis of the identification of pancreatic elastase.

By means of the systematic examination of stool samples which did not react to elastase 1 using the traditional ELISA method, it

was possible after electrophoretic separation to produce samples containing elastase. Further characterisation showed that these proteins were various iso-enzymes, which are obviously not identified by the antibodies in commercial tests. This enzyme is clearly subject to marked genetic polymorphism, which is also reflected in the specialist literature in the description of pancreas elastase 1, 2 and 3. Our own tests showed that at least two iso-enzymes can appear simultaneously. It was also possible to show that these elastases in their concentration dependence to pancreas damage reacted in exactly the same way as elastase 1.

In contrast to the familiar solutions, which are restricted to the specific identification of pancreas elastase 1, it was surprisingly discovered that by the identification of all the pancreas elastase iso-forms, of which pancreas elastases 1, 2 and 3 are currently known, the diagnostic relevance with regard to pancreatic function could be significantly improved. The invention therefore relates to a procedure for producing anti-elastic antibodies in the usual way. However, this procedure is characterised by the fact that the specific antigens used either singly or in combination represent all known elastase iso-enzymes or partial elements of such enzymes or cross-reacting synthetic sequences.

Partial elements are ideally obtained by means of peptide synthesis, with the amino-acid sequence being derived beforehand from the overall sequence by using structural analysis methods or alternatively being identified according to protein sequencing. Although the peptides alone can trigger antibody induction, it proved appropriate in terms of the invention to link these peptides to common carrier substances such as haemocyanine. It is possible with the peptides in the invention to produce monoclonal and polyclonal antibodies.

For the production of the polyclonal anti-peptide antibodies in the invention, animals such as rabbits, guinea pigs, goats, chickens or fish are immunised with the peptides in the familiar way. For the production of monoclonal antibodies, the peptides are used in the familiar way for the induction of specific B-cells, which after fusion with myelome cells produce hybridome cells, which then according to familiar cloning procedures are cultivated in cell lines which secrete specific monoclonal antibodies. The mono- or polyclonal antibodies in the invention react only with the used specific epitopes or with well-known elastase iso-enzymes.

It was shown that antibodies against the peptides A-V-K-E-G-P-E-Q-V-I-P-I-N, Y-T-N-G-P-L-P-D-K-L-Q-Q-A-R, R-S-G-C-N-G-D-S-G-G-P-L-N, G-P-L-N-C-P-T-E-D-G-G-W-Q, G-T-E-A-G-R-N-S-W-P-S-Q-I, H-N-L-S-Q-N-D-G-T-E-Q-Y-V, W-G-K-T-K-T-N-G-Q-L-A, V-S-S-R-G-C-N-V-S-R-K-P-T, G-G-E-E-A-R-P-N-S-W-P-W-Q, S-S-S-R-T-Y-R-V-G-L-G-R-H-N, K-D-W-N-S-N-Q-I-S-K-G-N-D, G-P-L-N-C-Q-A-S-D-G-R-W, G-A-L-P-D-D-L-K-Q-G-R-L, S-L-Q-Y-E-K-S-G-S-F-Y, F-G-C-N-T-R-R-K-P-T-V-F-T react highly specifically with the iso-forms of the pancreas elastase and do not react unspecifically with other stool components.

Another subject of the invention is the use of the elastase antibodies in the invention for the identification and quantification of al known elastase iso-enzymes in body fluids and in stool. The invention is therefore also relevant in terms of identification systems, particularly an immune-chemical identification system to establish the functionality of the pancreas as an aid to the recognition of functional disorders of this organ. For this purpose the specific antibodies can be connected to an suitable carrier adsorptively or chemically using familiar coupling procedures. Membranes or particles are suitable carriers. The Sandwich-ELISA of the invention can be used with cross-reactive epitope antibodies or a combination of various epitope antibodies to identify and to quantify pancreas elastase

in stool and in serum or plasma quickly and specifically.

It can also be used for the diagnosis or to establish the absence of pancreas involvement in abdominal complaints and exocrine pancreas insufficiency.

There are also cases in which the detection of elastase 1 is in itself sufficient to permit a certain diagnosis of pancreas disorders. In such instances, the procedure in the invention is carried out as follows:

The DNA-sequence for human elastase 1 (JP 1987000276-A/6) was transferred to the amino-acid sequences. With the aid of common protein structure programmes, it was possible to identify several amino-acid sequences that displayed a potential epitope structure. It was shown that antibodies against the peptides A-V-K-E-G-P-E-Q-V-I-P-I-N, Y-T-N-G-P-L-P-D-K-L-Q-Q-A-R, R-S-G-C-N-G-D-S-G-G-P-L-N and G-P-L-N-C-P-T-E-D-G-G-W-Q bind elastase 1 highly specifically and do not react unspecifically with other stool components.

The invention relates to a procedure for the production of anti-elastase antibodies in the usual way. The distinguishing characteristic is that the specific antigens used have previously been derived from the amino-acid sequence and chemically synthesised by methods of structural analysis. The invention also makes it possible to use parts of these synthetic peptides for the production of antibodies. Although the peptides alone trigger antibody induction, it has proven to be appropriate to connect these peptides to common carrier substances such as haemocyanine. It is possible to produce both monoclonal and polyclonal antibodies from the peptides used in the invention.
Animals such as rabbits, guinea pigs, goats, chickens and fish

were immunised with peptides in the usual way in order to produce the polyclonal antipeptide bodies in the invention. For the production of monoclonal antibodies, the peptides are used in the familiar way for the induction of specific B-cells, which after fusion with myelome cells generate hybridome cells, which according to familiar cloning procedures are cultivated in cell lines which secrete specific monoclonal antibodies. The mono- and polyclonal antibodies in the invention react only with the specific epitope used or with mature elastase 1.

A further subject of the invention is the use of the elastase 1 - epitope-specific antibodies for the identification and quantification of elastase 1 in body fluids and in stool. The invention is therefore also relevant to an immuno-chemical identification system for establishing the functionality of the pancreas as an aid to the identification of functional disorders of this organ. The specific antibodies can for this purpose be connected adsorptively or chemically to any suitable carrier using familiar coupling methods. Membranes or particles are suitable carriers. With the aid of the Sandwich-ELISA in the invention in conjunction in each case with two different epitope antibodies, it is possible to identify and to quantify elastase 1 in stool and in serum or plasma quickly and specifically.

Implementation example 1 - Production of specific anti-peptide antibodies which are targeted against definite segments of mature human Elastase 1.

Using the fixed-phase synthesis according to Merrifield, peptides with the amino-acid sequences NH_2-A-V-K-E-G-P-E-Q-V-I-P-I-N-COOH, NH_2-Y-T-N-G-P-L-P-D-K-L-Q-Q-A-R-COOH, NH_2-R-S-G-C-N-G-D-S-G-G-P-L-N-COOH and NH_2-G-P-L-N-C-P-T-E-D-G-G-W-Q-COOH were synthesised. By means of familiar procedures, the peptides are coupled with common limpet haemocyanine (KLH) -(1 mg peptide/mg KLH). In each case, 300 µg of this conjugate with the addition of a Freund adjuvant are used for the immunisation of a rabbit or a chicken. After three vaccinations, the animals are bled. After the serum is obtained, the specificity of the anti-serum is tested in an ELISA. For this purpose free peptide is adsorbed on to the surface of the cavities of microtitre plates. After the incubation of the cavities with the antiserums, they are thoroughly washed. Antigen-antibody reactions are detected in the usual way using an anti-rabbit or anti-chicken POD conjugate and TMB as a substrate. Every antiserum reacts only with the homologous peptide.

Implementation example 2 - Proof of the specificity of the antibodies in the invention

Elastase 1 specificity can be demonstrated in the Western blot. Here coarsely and highly purified elastase 1 from stool is separated from accompanying impurities by means of polyacrylamide gel electropheresis according to their relative molecular mass. The protein zones from the gel are transferred to nitro cellulose by means of a „Semi-dry-blotting" apparatus. After saturation of the free connection places of the membrane with re-suspended dried skimmed milk, the membranes are incubated with the anti-

peptide antiserums diluted at a rate of 1 : 500. After intensive washing of the membranes to remove all unspecifically-bound antibodies, the membranes are incubated with anti-rabbit antibodies marked with phosphatase. The specifically-bound secondary antibodies that remain on the membrane after washing are made visible after the substrate has been added. This shows that only elastase was identified in the sample used.

Implementation example 3 - Identification of elastase 1 in stool using the invention antibody in an ELISA

The elastase 1 in serum samples or in stool samples is identified in a fixed phase enzyme immuno-assay based on the sandwich technique. A polyclonal antibody that is targeted against epitopes of elastase 1 is dissolved in a carbonate/bicarbonate buffer solution pH 9,6 and placed in the wells of a microtiter plate. After incubation at 4 °C over 12 h, the non-bound antibodies are removed by washing with PBS. The still-free connection places of the carrier material are blocked by a PBS buffer containing ethanolamine and Tween 20. Blocking occurs over a 90 minute period at room temperature. After washing, the serum or stool samples diluted in PBS are placed in the wells via pipettes. The 60-minute incubation at room temperature is ended by washing. A second elastase 1-specific polyclonal antibody that is conjugated with biotin is added to the elastase that is connected to the first antibody.

After an incubation of 30 minutes and the washing process, the biotin-marked antibody with peroxidase-conjugated streptavidin is identified. The non-connected streptavidin is removed in the final washing phase. TMB is added as a substrate for the peroxidase and after a defined time the colour reaction is

stopped by the addition of HCl. The alteration in optical density is measured. The intensity of the colour reaction is proportional to the elastase 1 concentration of the sample.

Implementation example 4 - production of specific anti-peptide antibodies which are targeted against defined segments of isoforms of pancreas elastase.

Using fixed-phase synthesis according to Merrifield, the peptides are synthesised with the amino-acid sequences A-V-K-E-G-P-E-Q-V-I-P-I-N, Y-T-N-G-P-L-P-D-K-L-Q-Q-A-R, R-S-G-C-N-G-D-S-G-G-P-L-N, G-P-L-N-C-P-T-E-D-G-G-W-Q, G-T-E-A-G-R-N-S-W-P-S-Q-I, H-N-L-S-Q-N-D-G-T-E-Q-Y-V, W-G-K-T-K-T-N-G-Q-L-A, V-S-S-R-G-C-N-V-S-R-K-P-T, G-G-E-E-A-R-P-N-S-W-P-W-Q, S-S-S-R-T-Y-R-V-G-L-G-R-H-N, K-D-W-N-S-N-Q-I-S-K-G-N-D, G-P-L-N-C-Q-A-S-D-G-R-W, G-A-L-P-D-D-L-K-Q-G-R-L, S-L-Q-Y-E-K-S-G-S-F-Y, F-G-C-N-T-R-R-K-P-T-V-F-T. The peptides are coupled to common limpet haemocyanine (KLH) using the familiar procedure (1 mg peptide/mg KLH). In each case 300 µl of this conjugate with the addition of a Freund adjuvant are used for the immunisation of a rabbit or a chicken. After three vaccinations, the animals are bled. After obtaining the antibodies (purification via a protein A pillar or by fractional precipation), their specificity is tested in an ELISA. For this purpose free peptide is adsorbed on to the surface of the cavities of microtiter plates. After the incubation of the cavities with the homologous or heterologous antibodies, the cavities are thoroughly cleaned. The antigen-antibody reactions are detected in the usual way using anti-rabbit or anti-chicken POD conjugates. Every antibody reacts only with the homologous peptide.

Implementation example 5 - proof of the specificity of the

antibody in the invention

The specificity of the antibodies for various iso-forms of the pancreas elastase can be demonstrated in the Western blot. For this purpose coarsely and highly purified elastase samples from stool and pancreatic juice are separated from accompanying impurities by means of polyacrylamide gel electrophoresis according to their relative molecular mass. The protein zones from the gel are transferred to nitrocellulose with the aid of a "semi-dry" blotting apparatus. After saturation of the free connecting places of the membrane with re-suspended dry skimmed milk, the membranes are then incubated with the pre-diluted anti-peptide antibodies either alone or in various combinations. After intensive washing of the membranes to remove all unspecifically bound antibodies, the membranes with alkaline phosphatase-marked anti-rabbit antibodies are incubated in a previously established concentration. The specific secondary antibodies remaining on the membrane after washing are made visible after the addition of substrate. It was shown that elastase can be detected in all samples with individual antibodies or antibody mixtures but that not every antibody detects all iso-forms.

Implementation example 6 - Identification of the pancreas elastase in stool and serum using the antibodies in the invention

The elastase in serum, plasma or stool is identified using a fixed-phase ELISA based on the sandwich technique. For this purpose individual invention antibodies or a corresponding mixture of several of the invention antibodies are dissolved in a carbonate/bicarbonate buffer solution pH 9,6 and placed in the wells of a microtiter plate. After incubation at 4 °C, the non-bound antibodies are removed by washing with PBS. The remaining free connecting places of the carrier substance are blocked by an

ethanolamine/Tween 20-PBS buffer. Blocking takes place at room temperature over 90 minutes. After washing, the serum or stool samples diluted in PBS are placed in the wells via pipettes. The 60-minute incubation at room temperature is ended by washing. Individual invention antibodies or a mixture of several invention antibodies conjugated with biotin are used as detection antibodies. After a 30-minute incubation and the washing process, the biotin-marked antibody is identified with peroxidase-conjugated streptavidin. In the final washing step, the non-bound streptavidin is removed. Next the peroxidase concentration is established with TMB as a substrate. After the addition of HCl to end the enzyme reaction, the alteration of the optical density is measured. The intensity of the colour reaction is proportional to the elastase concentration in the sample.

amended claims (PCT Chapter II)
Patent Claims

1. Diagnostic procedure for identification of a disorder of the pancreas by determining the overall content of all known pancreatic elastases (iso-enzymes)in the serum, secretions or excretions of a patient.

2. Procedure according to claim 1, characterised by the fact that identification is by means of immuno-chemical systems using monclonal or polyclonal antibodies that can singly and specifically or cross-reactively recognise all elastase iso-enzymes with the amino-acid sequence Thr-Met-Val-Ala-Gly-Gly-Asp-Ile-Arg.

3. Procedure according to claim 2, characterised by the fact that the antibodies used are obtained by means of antigenes consisting of the complete elastases 1, 2 and 3 or of their sub-units. The amino-acid sequence Thr-Met-Val-Ala-Gly-Gly-Asp-Ile-Arg is not used as an elastase fragment or as an immunologically effective partial sequence thereof.

4. Procedure according to claim 3, characterised by the fact that the following synthetic peptides are primarily used as antigenes which after the immunisation of animals induce antibodies which cross-reactively recognise several elastases but do not concern the amino-acid sequence Thr-Met-Val-Ala-Gly-Gly-Asp-Ile-Arg:

NH2-A-V-K-E-G-P-E-Q-V-I-P-I-N-COOH
NH2-Y-T-N-G-P-L-P-D-K-L-Q-Q-A-R-COOH
NH2-R-S-G-C-N-G-D-S-G-G-P-L-N-COOH
NH2-G-P-L-N-C-P-T-E-D-G-G-W-Q-COOH
NH2-G-T-E-A-G-R-N-S-W-P-S-Q-I-COOH

NH2-H-N-L-S-Q-N-D-G-T-E-Q-Y-V-COOH
NH2-W-G-K-T-K-T-N-G-Q-L-A-COOH
NH2-V-S-S-R-G-C-N-V-S-R-K-P-T-COOH
NH2-G-G-E-E-A-R-P-N-S-W-P-W-Q-COOH
NH2-S-S-S-R-T-Y-R-V-G-L-G-R-H-N-COOH
NH2-K-D-W-N-S-N-Q-I-S-K-G-N-D-COOH
NH2-G-P-L-N-C-Q-A-S-D-G-R-W-COOH
NH2-G-A-L-P-D-D-L-K-Q-G-R-L-COOH
NH2-S-L-Q-Y-E-K-S-G-S-F-Y-COOH
NH2-F-G-C-N-T-R-R-K-P-T-V-F-T-COOH

5. Procedure according to claims 1 - 5, characterised by the fact that antibodies are used singly or in a combination in an immuno-chemical identification system.

6. Immunological test kits for the diagnosis and progress check of diseases of the pancreas using stool or body fluids containing one or more of the antibodies used in claims 2-5.

7. Procedure for obtaining monoclonal and/or polyclonal antibodies that react specifically with human elastase 1 in stool or body fluids and are induced by the usual immunisation procedures, characterised by the fact that the peptides A-V-K-E-G-P-E-Q-V-I-P-I-N, Y-T-N-G-P-L-P-D-K-L-Q-Q-A-R, R-S-G-C-N-G-D-S-G-G-P-L-N and G-P-L-N-C-P-T-E-D-G-G-W-Q or immunogenic partial peptides thereof are used as antigenes for the immunisation of vertrebrates, especially of small mammals and birds.

8. Procedure according to claim 7, characterised by the fact that before immunisation the free peptides are coupled with suitable carrier substances, primarily haemoczanine or albumin.

9. Procedure according to claims 7 and 8, characterised by the fact that polyclonal antibodies are produced using chickens as experimental animals.

10. Polyclonal antibodies, insofar as they were produced according to claims 7 to 9.

11. Monoclonal antibodies, insofar as they were produced according to claims 7 and 8.

12. Peptide A-V-K-E-G-P-E-Q-V-I-P-I-N

13. Peptide Y-T-N-G-P-L-P-D-K-L-Q-Q-A-R

14. Peptide R-S-G-C-N-G-D-S-G-G-P-L-N

15. Peptide G-P-L-N-C-P-T-E-D-G-G-W-Q

16. Purification and detection systems for human elastase 1, characterised by the fact that they contain at least one of the invention antibodies according to claims 10 and 11.

17. Immunological test kits for the diagnosis and progress check of diseases of the pancreas and of mucoviscidosis using stool or body fluids.

18. Immunological test kits according to claim 17, characterised by the fact that 2 different antibodies are used (Sandwich-ELISA).

Summary

A procedure is described for the identification of a functional disorder of the pancreas. In the invention, this goal is achieved by the use of parts of all iso-enzymes of the pancreas elastase and of synthetic amino-acid sequences as antigens for obtaining specific antibodies, as well as their use in immuno-chemical test procedures.

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If each inventor understands English, the Declaration and Power of Attorney below is suitable for use when filing a regular patent application and also when entering the national stage, in the case of an International application designating the USA under the PCT.

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION	Attorney Docket No. 101195

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name,
I believe I am the original, first and sole inventor (if only one name is listed below at 201) or an original, first and joint inventor (if plural names are listed below at 201-205) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Diagnostic Method for Detecting Disturbances of the Pancreas

the specification of which (check one)

____ is attached hereto

__✓__ was filed on _____3 Sept. 1999

under Serial Number __PCT/DE99/02816_ and was amended on _____20 Oct. 2000____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56.

I list below any prior foreign application(s) for patent or inventor's certificate in respect of which foreign priority benefits are claimed under 35 USC 119; and any prior foreign application(s) for patent or inventor's certificate in respect of which such foreign priority rights are not claimed and which has a filing date before that of any application in respect of which such foreign priority benefits are claimed:

Application Number	Country	Filing Date (day, month, year)	Priority Claimed under 35 USC 119
198 40 900.1	**Germany**	**8 Sept. 1998**	YES: ✓ NO:
199 23 892.8	**Germany**	**25 May 1999**	YES: ✓ NO:
			YES: NO:

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

Application No.	Filing Date

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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